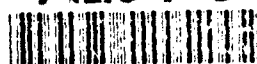


Naval Health Research Center

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**EFFECTIVENESS OF GLYCEROL INGESTION
FOR ENHANCED BODY WATER RETENTION
DURING COLD WATER IMMERSION**

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REPORT No. 90-33

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NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
BETHESDA, MARYLAND



**Effectiveness of Glycerol Ingestion for
Enhanced Body Water Retention
During Cold Water Immersion**

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Report No. 90-33 was supported by the Naval Medical Research and Development Command, Bethesda, Maryland, Department of the Navy, under research Work Unit No. 62233N MM33P30.02-6005. The opinions expressed in this paper are those of the authors and do not reflect official policy or position of the Department of the Navy, the Department of Defense, or the U.S. Government. The authors wish to thank the men of SEAL Delivery Team-ONE and the corpsmen of the Naval Health Research Center.

SUMMARY

The efficacy of ingesting an aqueous glycerol (GLY) solution to reduce diuresis and enhance body water retention during prolonged cold water dives was evaluated. Nine Navy divers [31 ± 5 years, height 175.0 ± 4.5 centimeters (cm), weight 78.9 ± 9.5 kilograms (kg), and 16 ± 2 percent (%) body fat] performed three-hour dives at 5 feet fresh water (FFW) maintained at 13°C . Divers wore thermal undergarments and dry suits and used a closed-circuit (MK-15) rebreathing unit. Six subjects were assigned to either a water treatment (WT) or glycerol treatment (GT) group. Three other divers performed a repeated measures design with dives separated by a day of nondiving. Statistical analyses of data from repeated and nonrepeated subjects consistently revealed no significant differences between and within these groups of subjects. Data were thus pooled into two treatment groups (WT = 6) and (GT = 6). During the predive period, divers drank approximately 2 liters of flavored water solutions [30 milliliters per kilogram (ml/kg) lean body mass (LBM)] with or without GLY (1.2 ml/kg LBM). Twelve SEAL Delivery Vehicle (SDV) operators performed a double-blind taste test to determine the acceptability of different concentrations of GLY solutions (with and without artificial sweetener). Solutions with artificial sweetener consistently scored lower; however, all solutions were considered "acceptable" if proven effective in achieving a hydration advantage. The total GLY ingested by each GT diver averaged 79.3 ± 8.2 ml. The volumes of all fluids ingested and excreted were measured. Blood samples were taken at prehydration (PRH), 90 minutes after hydrating (PH), and 20 minutes postdive (PD). Divers wore an external condom catheter connected to an 8 millimeter (mm) outside-diameter rubber tube that penetrated the dry suit. This tube was attached via a quick disconnect connector to a urine collection bag. Total urine output did not differ between treatments [WT = 2.1 ± 0.8 liters versus GT = 2.4 ± 0.5 liters]. Serum glucose (sGLU) and serum lactate (sLAC) did not differ between treatments or times, but sLAC was significantly lower ($P < 0.02$) for both treatments at PD than at PH. Serum free fatty acids (sFFA) at PD for both treatments were significantly higher ($P < 0.2$) than at PRH and PH. Serum glycerol (sGLY) values for GT were 200 times above PRH at PH and 100 times above PRH at PD. The amount of urinary glycerol (uGLY) collected during the hyperhydration period (PRH to PH) and the three-hour dive periods accounted for

4.1% and 10.3%, respectively, of the total GLY ingested. Urine electrolyte values did not differ between treatments, except GT lost significantly ($P < 0.02$) more sodium (Na) [53.7 ± 9.9 versus 21.6 ± 6.2 milliequivalent per liter (meq/l)] during the PH period. Hyperhydration with GLY (1.2 ml/kg LBM) mixed in 2 liters of water and consumed over 30 minutes appears ineffective in significantly reducing body water loss in divers under the stress of prolonged cold water immersion. It may be that altering the timing and/or concentration of the GLY doses will produce a significant effect under these test conditions.



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INTRODUCTION

In humans, water immersion, whether head-out or whole body, represents a condition of environmental stress which elicits unique physiologic responses (Epstein 1978; Greenleaf 1984). Immersion has been shown to significantly increase diuresis (Claybaugh et al. 1986; Deuster et al. 1989; Epstein 1978; Harrison et al. 1986; Rochelle and Horvath 1978). Accompanying the increased urine production is an increased loss of sodium (Na) and potassium (K) ions (natriuresis and kaliuresis) (Epstein et al. 1975; Epstein 1978). These physiologic responses occur when subjects are immersed in either cold (Deuster et al. 1989; Doubt et al. 1988; Young et al. 1987) or thermoneutral water (Epstein 1978; Greenleaf et al. 1980; Greenleaf et al. 1981; Greenleaf et al. 1983; McCalley 1964). Exposure to cold air alone significantly increases diuresis and plasma volume loss (Young et al. 1987); however, cold water immersion produces a more powerful stimulus (Deuster et al. 1989; Trippodo et al. 1983).

It is generally accepted that the underlying mechanism(s) of immersion diuresis involve neural, hormonal, and hemodynamic factors. A primary factor appears to be the redistribution of blood [approximately 700 milliliters (ml)] into the thoracic region and a concomitant increase in right atrial and pulmonary transmural pressure (Greenleaf 1984). This, in turn, stimulates atrial receptors and the release of natriuretic and diuretic "factors," e.g., atrial natriuretic factor (ANF) (Gauer et al. 1970; Gupta et al. 1982; Trippodo et al. 1983).

Prolonged immersion diuresis, either with or without supplemental water intake, produces significant plasma volume loss over a range of 8% to 18% and whole body dehydration (Bøning et al. 1972; Deuster et al. 1989; Doubt et al. 1988; Epstein 1978; Harrison et al. 1986; Rochelle and Horvath 1978; Goforth, unpublished data). Dehydration (Buskirk et al. 1958; Jacobs 1980), hypohydration (Caterisano et al. 1988; Caldwell et al. 1984), and plasma volume loss negatively affect work capacity (Coppin et al. 1978; Craig and Cummings 1966; Saltin 1964). Cold exposure (10°C to 18°C) compounds the problems associated with immersion diuresis by causing loss of muscular power and strength (Bergh 1980; Caterisano

et al. 1988; Gilmour and Edwards 1986), loss of mental faculties (Vaughan 1975; Vaughan 1977), and possible death due to severe hypothermia.

An obvious strategy to overcome the large fluid volume loss during water immersion would be to hyperhydrate the subject. However, since the kidneys depend on adequate vascular pressures and volume to filter the blood, an increased vascular volume becomes a stimulus for increased diuresis (Epstein 1978). Therefore, forced hydration with water before and during cold and thermoneutral immersion tests has proved unsuccessful in compensating for diuresis and preventing dehydration (Deuster et al. 1989; Doubt et al. 1988; Epstein 1978; Harrison et al. 1986; Goforth, unpublished data). Another strategy has been to infuse plasma volume expanders (e.g., albumin, dextran, or saline) (Coyle et al. 1966; Epstein et al. 1975; Hubbard et al. 1984; Kanstrup and Ekblom 1982; Kanstrup and Ekblom 1984). Infusion of albumin is reported to acutely increase plasma oncotic pressure and maintain an elevated plasma volume in the face of a heat stress challenge (Hubbard et al. 1984). The application of this procedure has not been tested with divers during cold water immersion. However, this sort of invasive technique would have only limited applications for military divers.

Ingesting an aqueous glycerol (GLY) solution has also reportedly been successful in enhancing plasma volume retention of subjects exercising in a hot environment (Lyons et al. 1987a; Lyons et al. 1990). Glycerol readily equilibrates with the extracellular space (Lin 1977; Tourtellotte et al. 1972) (estimated at 50% to 65% of the body weight) and is reported to enhance body water retention in thermoneutral (Lyons et al. 1987b; Riedesel et al. 1987) and hot (Lyons et al. 1987a; Lyons et al. 1990) environments. Glycerol ingestion is an appealing intervention because of its noninvasive administration and its reported ability to temporarily sequester water in the interstitial spaces (Lin 1977; Tourtellotte et al. 1972). Glycerol is an endogenous triose and, as a metabolic intermediate, has several metabolic paths, resulting in an estimated biochemical half-life of 30 to 60 minutes (Tourtellotte et al. 1972). Glycerol has the advantage of being highly miscible and can be easily administered orally or intravenously in large volumes (Tourtellotte et al. 1972). Furthermore, it is readily absorbed by the gastrointestinal tract, reaching peak serum values

in 60 to 90 minutes (Tourtellotte et al. 1972), and equilibrates rapidly across most nonneurological tissues (Tourtellotte et al. 1972). Since hyperhydration with GLY had been reported effective in retarding diuresis and plasma volume loss in terrestrial studies (Lyons et al. 1987a; Lyons et al. 1987b; Lyons et al. 1990; Riedesel et al. 1987), it appears to merit serious consideration for retarding diuresis during cold water immersion. Therefore, the purpose of this study was to examine the efficacy of ingesting GLY to enhance body water retention and reduce diuresis during a prolonged (three hour) cold water dive. The present study is part of a larger research program to develop interventions to attenuate the debilitating effects of prolonged cold water immersion, specifically upon the physical and cognitive performance of Navy divers wearing dry suits and thermal undergarments.

METHODS

Subjects

Nine Naval Special Warfare divers (SEALs) assigned to SEAL Delivery Vehicle (SDV) Team One [age 31 ± 5 years, height 175.0 ± 4.5 centimeters (cm), total body mass (TBM) 78.9 ± 9.5 kilograms (kg), lean body mass (LBM) 66.1 ± 6.9 kg, and body fat $16 \pm 2\%$] volunteered to participate in this study. Individual anthropometric data are provided in Table 1. Body fat was estimated according to the skinfold technique of Durnin and Wommersley (1974). Subjects were asked to refrain from using nicotine products and caffeinated beverages for 24 hours before and throughout the three-day experimental period. A full explanation of the risks, benefits, and research procedures was given to each subject prior to signing a consent form.

TABLE 1. ANTHROPOMETRIC MEASUREMENTS OF U.S. NAVAL SPECIAL WARFARE DIVERS (SEALS)

| Subject | Age (yr) | Height (cm) | TBM (kg) | LBM (kg) | Fat (%) |
|-----------|-------------|----------------|-------------|-------------|------------|
| Wi | 30 | 170.2 | 80.3 | 66.8 | 17 |
| Ce | 28 | 172.7 | 74.8 | 62.7 | 16 |
| Hn | 28 | 172.7 | 69.2 | 58.1 | 16 |
| Tt | 27 | 177.8 | 77.6 | 67.0 | 14 |
| Le | 28 | 175.3 | 88.0 | 73.7 | 16 |
| Ve | 32 | 167.6 | 66.8 | 55.7 | 17 |
| Ve | 43 | 177.8 | 78.6 | 66.0 | 16 |
| Bn | 29 | 180.3 | 77.1 | 67.2 | 13 |
| Lr | 31 | 180.3 | 98.2 | 77.6 | 21 |
| \bar{x} | 31 | 175.0 | 78.9 | 66.1 | 16 |
| S.D. | ± 5 | ± 4.5 | ± 9.5 | ± 6.9 | ± 2 |

Data Analyses and Treatment Groups

Six subjects were randomly assigned to a water control (n = 3) or a GLY experiment group (n = 3). The remaining three subjects volunteered to perform a repeated-measures design experiment (first GLY, then water), with each treatment separated by a day of rest. The Mann-Whitney t-test showed no significant differences among repeated subjects on any pertinent variable (e.g., body weight, blood, or urine metabolites) prior to the water or GLY treatment. Additionally, the Wilcoxon sign rank test revealed no significant differences between treatment responses of the repeated and nonrepeated subjects. Therefore, the data were pooled to form two treatment groups [water treatment (WT) = 6 and GLY treatment (GT) = 6]. The Mann-Whitney and Wilcoxon tests were then applied to these data to determine differences between the two treatment groups. Significance levels are reported at the < 0.05 level. Tabular and graphic representations of these data are expressed as means of six observations per treatment group.

Test Solutions

To evaluate the taste acceptability for different concentrations of GLY solutions (with and without artificial sweetener), 12 SDV operators performed a double-blind taste test. Acceptability of 4 concentrations (0.6, 0.9, 1.2,

and 1.5 ml GLY/kg LBM) representing 50%, 75%, 100%, and 125%, respectively, of the GT solution revealed a wide range of preferences (Figure 1). There was no clear preference for any GLY concentration. An equal number of subjects rated the most concentrated (125%) and most dilute (50%) as "best." Solutions containing artificial sweetener were not preferred and, in fact, were consistently rated "worst." However, all solutions were rated by subjects as "acceptable," meaning they would drink it for special missions if proven effective in achieving hyperhydration. The 100% solution (i.e., a concentration reported safe and effective in previous studies) was selected for the present study.

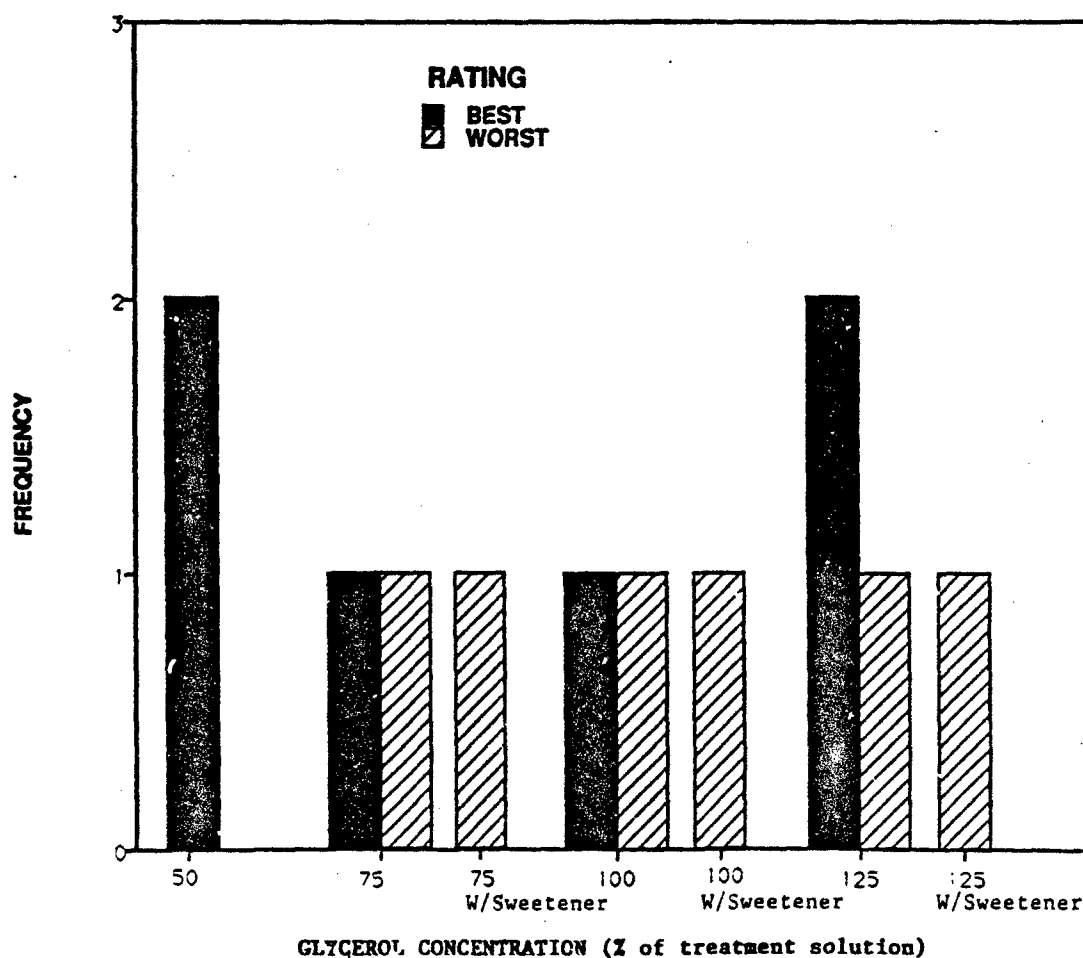


FIGURE 1. Acceptability ratings by SEAL Delivery Vehicle operators (n = 12) of different glycerol solutions with and without artificial sweetener.

Experimental Protocol

All subjects arrived at the dive facility, after fasting 12 to 16 hours, and sat for 20 to 30 minutes before having a baseline blood sample taken. Next, a high (78%) carbohydrate (CHO) meal was consumed by all subjects to minimize differences in nutritional status. This was designed to ensure sufficient substrate (glucose) for liver glycogen repletion and to minimize liver uptake of the ingested GLY, a gluconeogenic substrate. The meal was consumed 2 to 3 hours before the dive and contained 686 to 833 kilocalories (kcal), comprised of 140 to 160 grams (g) CHO, 12 to 18 g fat and 5 to 8 g protein.

After completing the meal, subjects were requested to void their bladder before commencing the hydration phase of the experiment. Subjects were then weighed (± 0.1 kg) on an electronic digital scale (West Scales). From this point on, the volume/weight of all urine/feces excreted and fluids ingested was carefully measured and recorded.

Subjects ingested individually determined volumes of either the aqueous GLY or control solution that contained 30 ml water/kg LBM (approximately 2 liters). The control solution, approximating the GLY solution in flavor and color, was made by adding 2 teaspoons saccharine and 2 tablespoons concentrated orange juice to the water. Only the orange juice was added to the GLY solution. The GLY treatment solution was individually calculated as 1.2 ml/kg LBM and averaged 79.3 ± 8.2 ml. This amount of GLY is considered a safe medical dosage (Tourtellotte et al. 1972) and is within the range used by other researchers (Lyons et al. 1987a; Lyons et al. 1987b; Lyons et al. 1990; Riedesel et al. 1987).

Equipment

Divers were outfitted with dry suits and a passive thermal protection system composed of polypropylene underwear (Thermax) and a Thinsulate-400 suit (3M Corporation) as undergarments. Additionally, they wore a wet suit hood and gloves made of 1/4-inch-thick neoprene. Divers remained in a sitting position at a depth of 5 feet in fresh water maintained at $13^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Closed-circuit rebreathing units (MK-15) with a carbon dioxide scrubber were used. External urinary catheters (Hollister) were worn as part of an overboard urine discharge system that exited the dry suit via an 8-millimeter- (mm) diameter tube. For

the purpose of collecting urine samples, the exit tube was fitted with a quick-release connector attached to a 1-liter collection bag.

Blood and Urine Collection and Analysis

Blood was sampled three times from the cubital vein. The first sample [prehydration (PRH)], taken 20 minutes after arriving at the dive tank, was used to establish the fasting baseline values for metabolites and electrolytes. The second sample [posthydration/predive (PH)], taken 60 minutes after completing the hydration protocol, was used to establish a PH baseline to compare with the postdive (PD) values. The third sample (PD) was taken 20 minutes after divers had exited the water, had removed their diving equipment, and had been sitting quietly for 20 to 30 minutes. To document metabolic changes, serum samples were analyzed for glucose, GLY, free fatty acids (FFA), lactate, and the following electrolytes: Na, K, calcium (Ca), chloride, creatinine, and phosphate. Hematocrit was determined from heparinized whole blood in triplicate using a microhematocrit centrifuge. Serum free fatty acids (sFFA) were analyzed using the colorimetric method of Novak (1965). Glycerol concentrations were determined in serum and urine samples using the spectrophotometric enzyme assay of Kreutz (1962). Lactate concentrations were determined from serum samples using the YSI Model 23L Lactate Analyzer (Yellow Springs Instruments, Inc.). Triplicate samples were run randomly on serum samples to check the reliability of the analyzer. Recalibration of the analyzer against a standard was performed initially, after each sample run, and regularly after each fifth sample determination.

Urine samples were obtained hourly by exchanging the collection bags attached to the diver's overboard urine discharge tube. Sample volumes were measured and subsamples refrigerated until analyzed for GLY, Na, K, Ca, chloride, creatinine, phosphate, osmolality, and specific gravity.

RESULTS

The volume of water ingested by the subjects in the GLY and water trials was $2,034 \pm 92$ ml and $1,918 \pm 78$ ml, respectively. The mean volume of GLY in the ingested solution was 79 ± 8 ml. The mean nutritional values for the predive

meals consumed by each treatment group did not differ significantly in kcals, CHO, fat, or protein.

Serum Data

Glucose: There were no significant differences in mean serum glucose (sGLU) levels of the water or GLY group at each sampling time (Figure 2). Likewise, within a given treatment (GLY or water), there were no significant differences in serum glycerol (sGLY) values between the three sampling times. All subjects maintained normal blood glucose concentrations of 5.24 to 5.63 millimolars (mM) or 94 to 101 milligrams per deciliter (mg/dl) throughout the three-hour experimental dive.

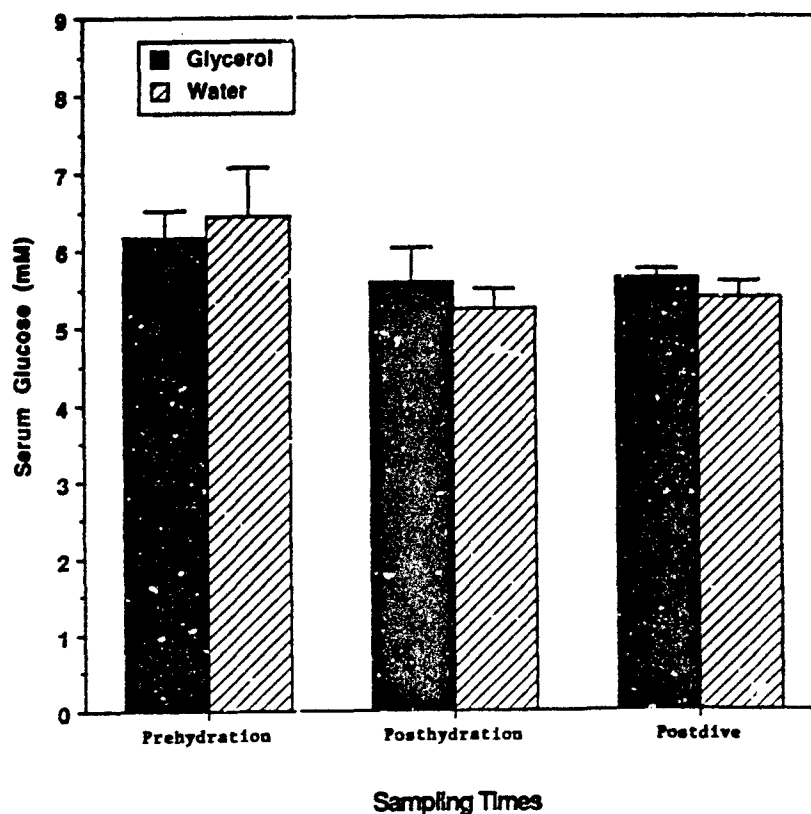


FIGURE 2. SERUM GLUCOSE. Values are expressed as means of six observations \pm S.E.M.

Free Fatty Acids: Concentrations of sFFA at PD, for both the water and the GLY trials, were significantly higher ($P < 0.02$) within comparable trials than at the PRH and PH times (Figure 3). Postdive sFFA concentrations were 63% and 65% higher than PRH values in the water and GLY trials, respectively. Additionally, the initial PRH sFFA values of subjects in the water group were significantly higher ($P < 0.001$) than in the GLY group. The sFFA values of the water trial group remained higher at PH and PD sampling times, but not significantly higher than that of the GLY group.

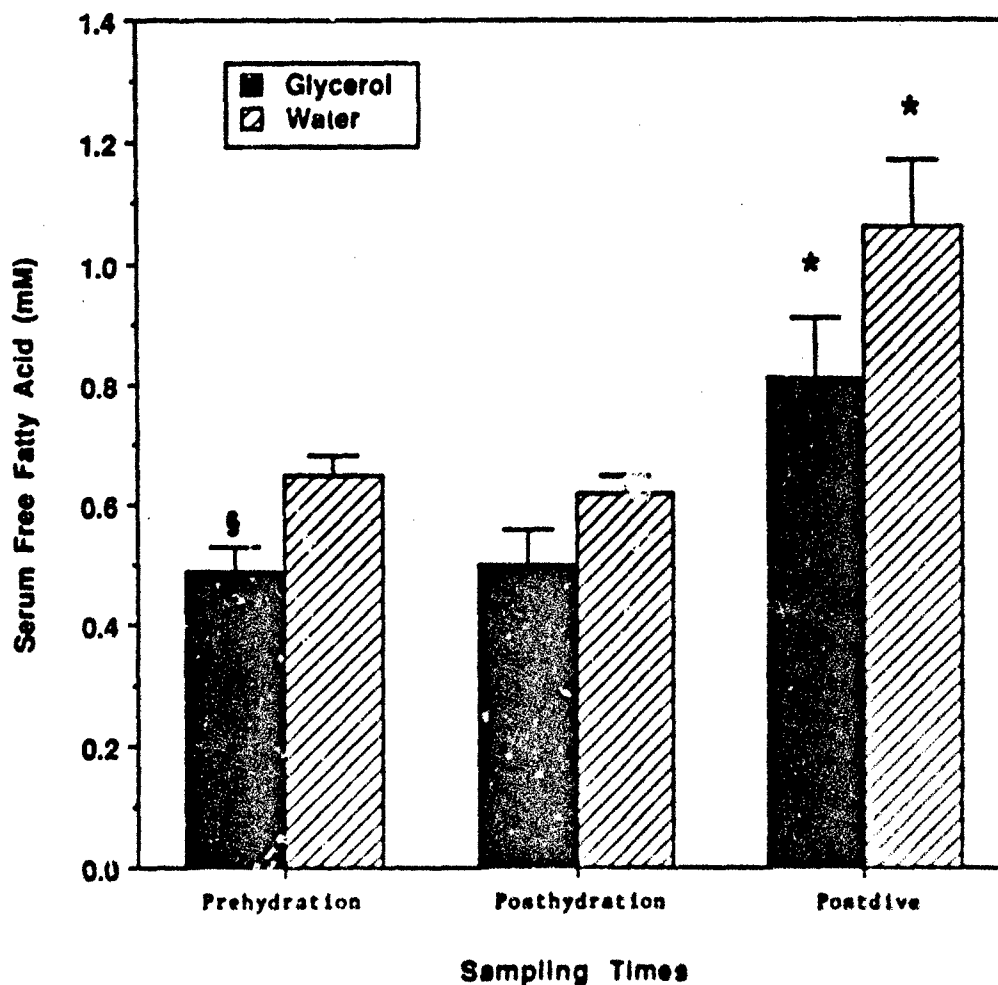


FIGURE 3. SERUM FREE FATTY ACIDS. Values are expressed as means of six observations \pm S.E.M. *Significant difference ($P < 0.05$) from prehydration and posthydration sampling times within the same treatment group. §Significant difference ($P < 0.05$) from corresponding water value.

Lactate: Concentrations of serum lactate (sLAC) for the water and GLY subjects were not significantly different at any of the three sampling times (Figure 4). However, both water and GLY groups demonstrate a significantly lower ($P < 0.02$) sLAC concentration at the PD time compared to the PH time. Mean sLAC values for all subjects throughout the study were within the normal range of 1.0 to 2.4 mM for nonexercising subjects.

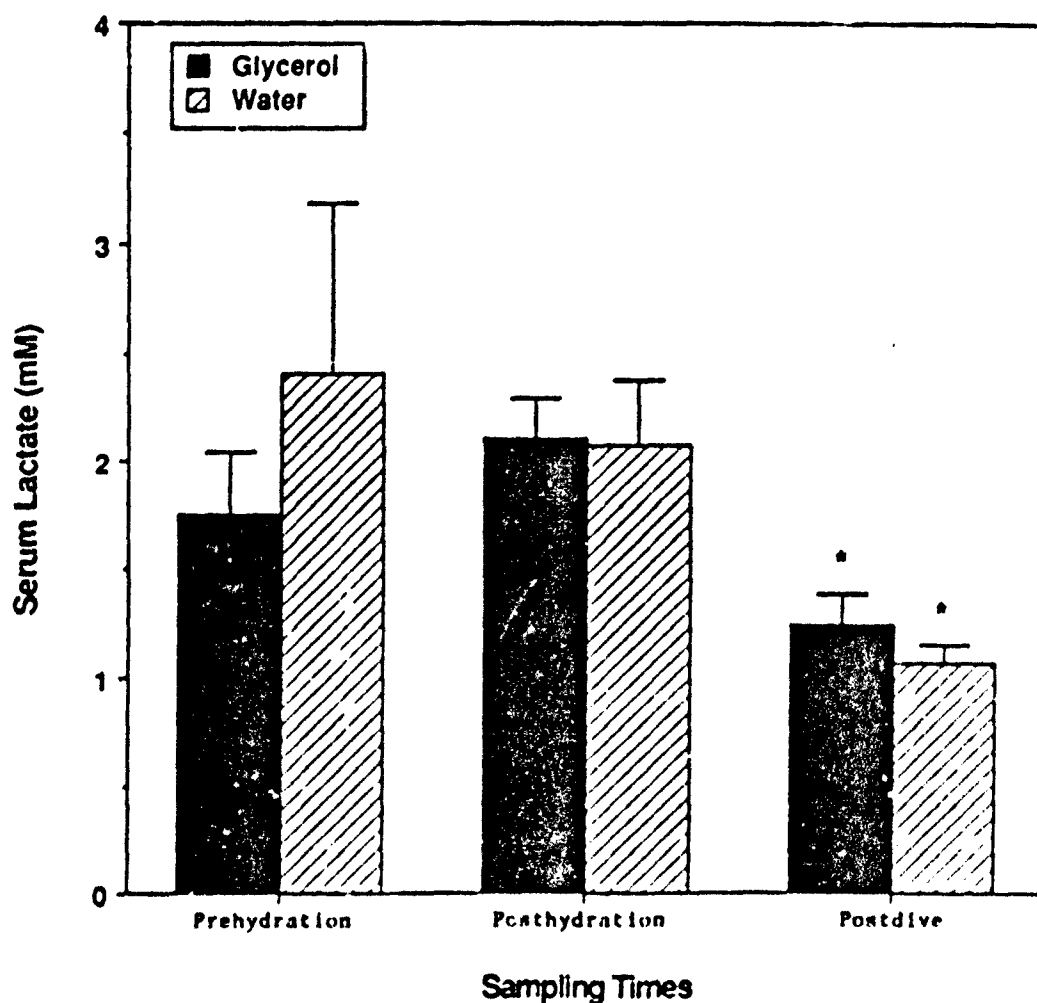


FIGURE 4. SERUM LACTATE. Values are expressed as means of six observations \pm S.E.M. *Significant difference ($P < 0.05$) from posthydration values within the same treatment.

Glycerol: At the PRH time period, the sGLY was 0.05 mM for all subjects (Figure 5). For the water trial group, the sGLY values remained level throughout the experiment. As expected, the mean sGLY values for the GLY group increased 200-fold above baseline at PH and was still approximately 100-fold at PD.

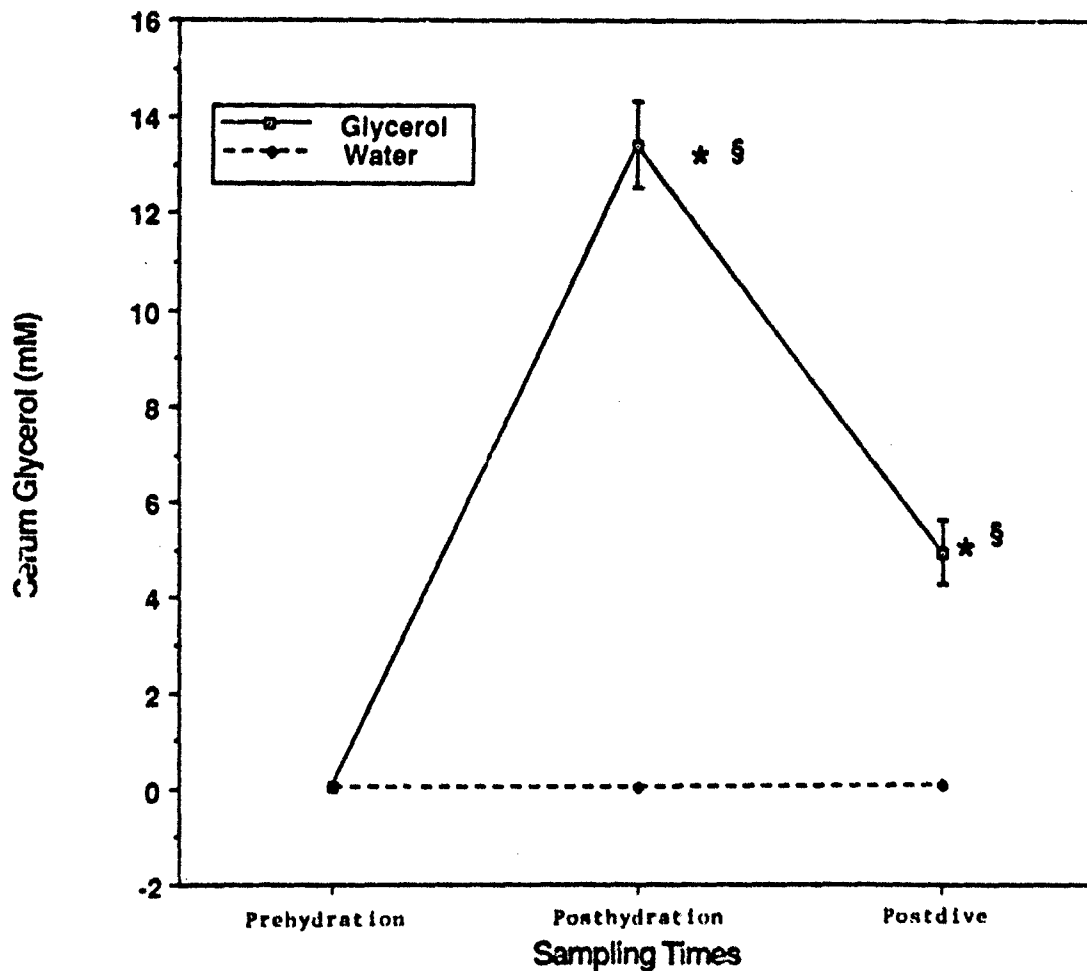


FIGURE 5. SERUM GLYCEROL. Values are expressed as means of six observations \pm S.E.M. *Significant difference ($P < 0.05$) from prehydration values. \$Significant difference ($P < 0.05$) from values of the water trials at the corresponding sampling time.

Serum Electrolytes: Serum electrolytes [Na⁺ and K⁺] were not significantly different between treatments and across all sampling periods (Table 2).

TABLE 2. HEMATOLOGICAL, SERUM, AND URINE DATA WITH AND WITHOUT VARIOUS DOSES OF GLYCEROL. *Significant difference ($P < 0.002$) between glycerol and water total excreted milliequivalent urine sodium at the posthydration time period. §Significantly higher ($P < 0.05$) hematocrit than posthydration hematocrit in the glycerol subjects.

| | Post-Hydration | Post-Dive |
|-------------------------|----------------|---------------|
| Urine | | |
| No Glycerol | | |
| Na ⁺ (meq) | 21.63 ± 15.10 | 68.72 ± 50.24 |
| K ⁺ (meq) | 11.13 ± 9.01 | 12.30 ± 7.70 |
| Cl ⁻ (meq) | 26.36 ± 22.00 | 50.60 ± 26.66 |
| Cr (meq) | 37.67 ± 32.03 | 35.21 ± 7.37 |
| Phos (meq) | 12.82 ± 7.85 | 13.54 ± 6.38 |
| Ca ²⁺ (meq) | 3.99 ± 3.13 | 5.71 ± 1.91 |
| Specific Gravity | 1.01 ± 0.01 | 1.01 ± 0.00 |
| Glycerol | | |
| Na ⁺ (meq) | *53.67 ± 24.18 | 66.75 ± 22.23 |
| K ⁺ (meq) | 23.54 ± 10.83 | 16.53 ± 8.03 |
| Cl ⁻ (meq) | 64.47 ± 42.03 | 71.38 ± 22.12 |
| Cr (meq) | 53.05 ± 27.64 | 32.96 ± 9.01 |
| Phos (meq) | 19.05 ± 8.27 | 14.46 ± 4.86 |
| Ca ²⁺ (meq) | 7.31 ± 4.97 | 6.34 ± 2.36 |
| Specific Gravity | 1.01 ± 0.01 | 1.01 ± 0.00 |
| Serum | | |
| No Glycerol | | |
| Na ⁺ (meq/L) | 137.70 ± 1.47 | 139.50 ± 1.71 |
| K ⁺ (meq/L) | 4.05 ± 0.17 | 3.95 ± 0.20 |
| Glycerol | | |
| Na ⁺ (meq/L) | 137.50 ± 1.47 | 139.30 ± 0.98 |
| K ⁺ (meq/L) | 4.12 ± 2.45 | 4.03 ± 0.22 |
| Hematocrit | | |
| No Glycerol | 41.33 ± 0.73 | 42.57 ± 0.73 |
| Glycerol | 39.65 ± 1.00 | §43.47 ± 0.79 |

Hematocrit: Hematocrits of GLY subjects increased significantly ($P < 0.05$) during the dive, possibly due to the transient, non-significant hemodilution observed at PH (Figure 6). Unfortunately, the more critical parameter, plasma volume, could not be calculated (Dill and Costill 1974) because hemoglobin concentrations were not obtained due to technical difficulties.

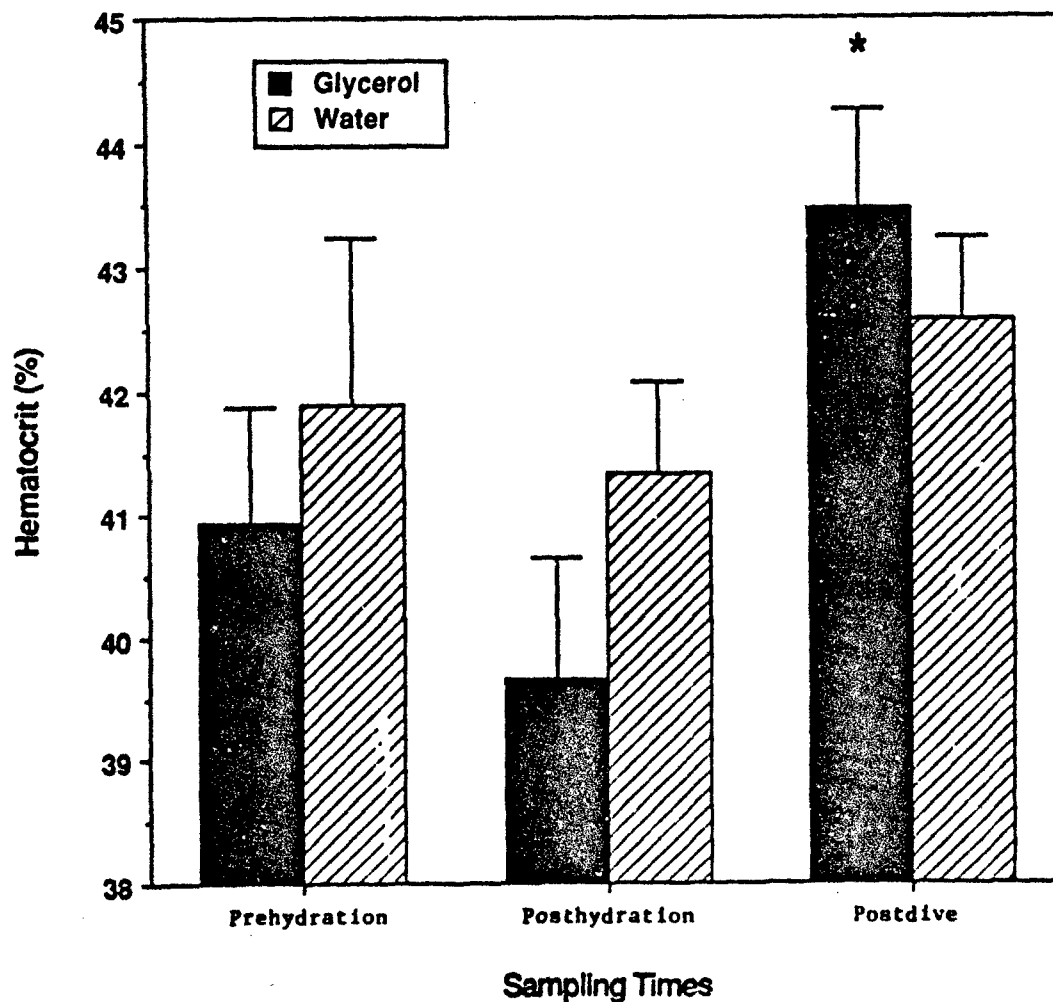


FIGURE 6. HEMATOCRIT PERCENTAGE. Values are expressed as means of six observations \pm S.E.M. *Significant difference ($P < 0.05$) from prehydration and posthydration values in the glycerol subjects.

Urine Data

Water Retention/Urinary Output: Urine output, total body weight loss, and nonurine weight loss during PH and dive sampling periods were not significantly different between treatment groups (Table 3). However, the total body weight loss and nonurine weight loss were consistently, but not significantly, lower in the GT group. During the dive, the GT group retained an average of 310 ml more water than the control group. This difference was a result of a smaller nonurinary water loss (0.45 ml versus 0.82 ml) exhibited by the GLY group (i.e., respiratory and sweat).

TABLE 3

| WATER AND BODY WEIGHT CHANGES DURING GLYCEROL AND WATER TREATMENTS | | | | |
|--|---------------------|--------------------|---------------|---------------|
| | Fluid in (liters) | Urine Out (liters) | | |
| | Hydration Volume | PH | Dive | Total |
| Water | 1.98 ±0.19 | 0.78 ±0.28 | 1.30 ±0.59 | 2.08 ±0.79 |
| Glycerol | 2.06 ±0.18 | 1.03 ±0.68 | 1.36 ±0.44 | 2.39 ±0.48 |

| WEIGHT LOSS DURING DIVE | | | | |
|-------------------------|-----------------|--------------------------------|-----------------|---------------|
| | Urine Weight | Weight Loss Nonurine Weight | Total Weight | |
| Water | 1.30 ±0.59 | 0.82 ±0.30 | 2.12 ±0.40 | 0.03 ±0.01 |
| Glycerol | 1.36 ±0.44 | 0.45 ±0.21 | 1.61 ±0.53 | 0.02 ±0.01 |

No significant difference at $P < 0.05$. Values are expressed as means of six observations \pm S.E.M.

Urine Glycerol: Baseline uGLY values of 0.05 mM were established for all subjects from urine collected upon arrival at the dive facility. The GLY concentrations in all subsequent urine samples (samples 2 through 5) were significantly greater ($P < 0.001$) in the GLY group than in the control group at the same sampling periods (Figure 7). The amount of GLY excreted during the 1.5-hour PH period represented only 4.1% of the total GLY ingested, while an additional 10.2% was excreted during the three-hour dive. During the hour following ingestion, uGLY increased rapidly and remained elevated throughout the dive.

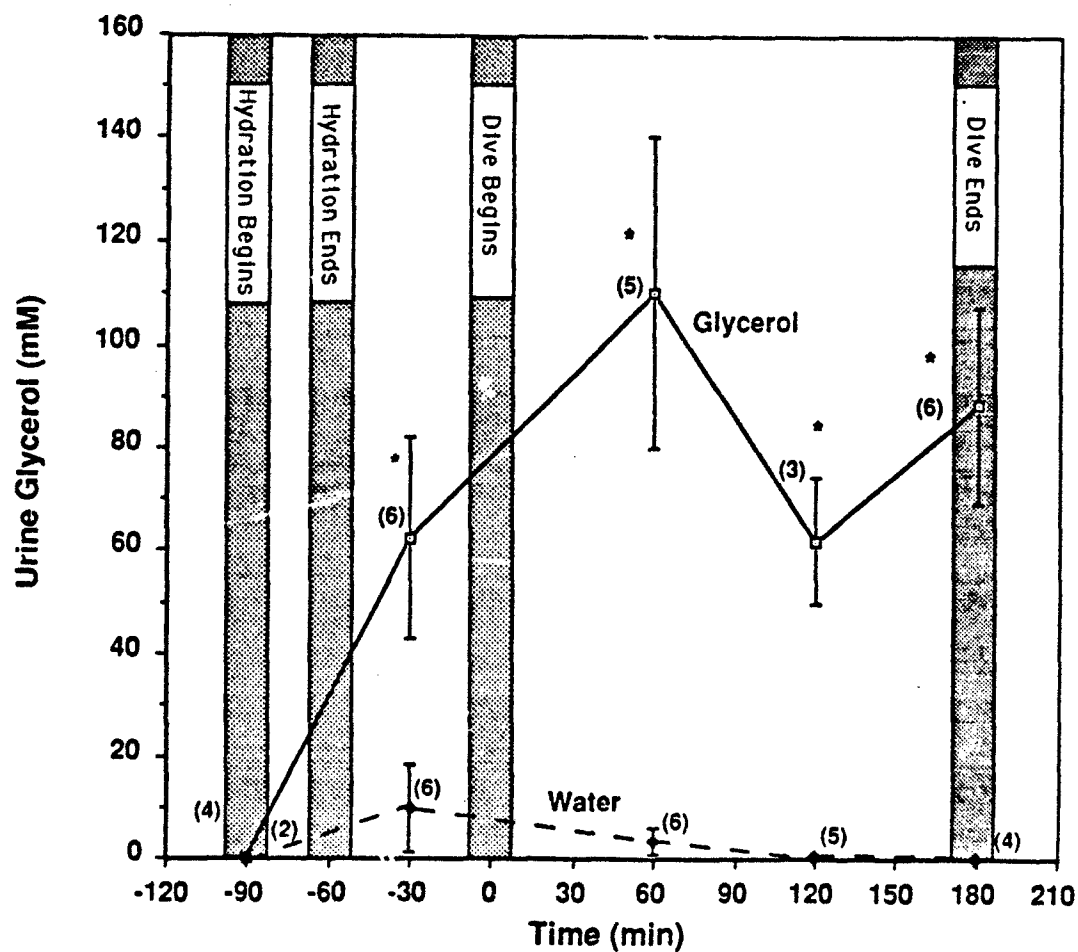


FIGURE 7. URINE GLYCEROL. Values are expressed as means \pm S.E.M. Number of observations is indicated in parentheses by each sampling time point. *Significant difference ($P < 0.05$) from corresponding values in the water trial group.

Urine Electrolytes: Urinary electrolyte data (see Table 2) did not differ significantly between treatments or within treatments across all sampling periods (except total meq Na excreted during the PH period). Glycerol subjects excreted significantly more ($P < 0.02$) Na than the water control group during the 60-minute PH period. However, during the dive, both treatment groups lost equivalent amounts of urinary Na (67 to 69 meq) and other electrolytes. During the six-hour experimental protocol, divers lost a total of 90 to 120 meq Na and 23 to 40 meq K.

DISCUSSION

Water Retention

Riedesel et al. (1987) reported a mean water retention of 400 ml of water after subjects ingested a GLY dose of 1.5 g/kg TBM with 1.5 liters of water and rested for 4 hours in a thermoneutral environment. In the present study, divers ingesting a slightly smaller GLY dose of 1.2 g/kg LBM had a mean water retention of 310 ml greater than the water controls. Given the 0.3 g difference in the GLY doses used in the present study, the relative amount of water retained is quite similar in the two studies. The amount retained lacked significance primarily because of the large between-subject variation in immersion diuresis. Claybaugh et al. (1986) found physically trained subjects to exhibit less diuresis and natriuresis during head-out immersion than untrained subjects. Physical fitness appears to blunt the diuretic response to increased thoracic volume caused by whole body or head-out immersion. Additionally, Rochelle and Horvath (1978) found that surfers who were chronically exposed to cold water exhibited a smaller plasma volume loss during cold stress than controls. However, cold acclimatization (habituation) was recently reported not to influence the subject's diuresis or plasma volume loss (Young et al. 1987). It is possible that a follow-up study matching the degree of physical condition and cold acclimation of the subjects may reduce individual variation and show a significant difference in water retention. It may also be necessary to alter the timing and concentration of the GLY doses to achieve a significant effect during cold water immersion.

Serum Metabolites

Cold exposure enhances the release of norepinephrine, increases blood GLY concentrations of GLY and FFA, and stimulates increased lipid oxidation (Smith et al. 1990; Therminarias et al. 1989; Wilson et al. 1969). Since sGLY uptake or removal is directly proportional to its concentration (i.e., the linear regression line passes through the origin), it is thought to be a better serum indicator of fat mobilization than sFFA (Wilson et al. 1969; Winkler et al. 1969). Resting humans exposed to 0°C air increased their plasma GLY concentrations by 88% within 3 hours (Wilson et al. 1969). Vallerand and Jacobs (1989) observed that resting humans exposed to 10°C air increased their metabolic rate 2.5 times, while fat and CHO oxidation increased an estimated 63% and 588%, respectively. Humans exercising [at 50% and 60% aerobic capacity ($\text{VO}_{2\text{ max}}$)] increased sGLY significantly more at 22°C than at 0°C air (Sink et al. 1989). These authors concluded that temperature and exercise act independently upon sGLY levels.

Peak sGLY concentrations are reported to occur within 60 to 90 minutes following ingestion (Frank et al. 1981). In the present study, sGLY increased from 0.053 mM to a peak of 13.4 mM at 90 minutes after ingesting a 1.2 g/kg LBM of a GLY solution. Riedesel et al. (1987) administered a similar GLY dosage to resting humans in a thermoneutral environment and reported peak sGLY values of 14.7 mM at 120 minutes postingestion. Gleeson et al. (1986) measured peak blood GLY values of 16.8 mM at 30 minutes postingestion of a 1.0 g GLY/kg LBM solution. After an exhaustive cycle exercise bout (86 minutes), the blood GLY values of these subjects had decreased 34.5% to only 11.0 mM, while exercising control cyclists had increased blood GLY values 580% to 0.29 mM. Miller et al. (1983) conducted a similar study using the same GLY dosage and observed a peak in blood GLY (13.0 mM) at 30 minutes postingestion.

In the present study, sGLY levels decreased 63% to 4.9 mM after a three-hour cold water immersion dive. This represented an estimated total body clearance rate of 0.035 mM GLY/minute. The liver is considered the organ primarily responsible for 75% of the GLY clearance, the remainder being removed from the plasma by the kidneys and the movement of GLY into extravascular spaces (Lin 1977). A two- to three-fold intersubject variation in GLY clearance has

been reported for humans (Frank et al. 1981). Riedesel et al. (1987) found that sGLY concentrations had decreased by only 29% (4.3 mM) four hours after ingesting the GLY solution. This represents an estimated total body clearance rate of only 0.018 mM GLY/minute, or half the rate observed in the present study. Exposure to cold air was reported to increase fat oxidation by 63% and, likewise, the turnover rate of both FFAs and GLY (Vallerand and Jacobs 1989). The cold conditions of our study may explain the two-fold difference in GLY clearance rates compared with Riedesel's. This difference may result from the increased diuresis associated with cold immersion resulting in a greater relative and absolute amount of renal clearance of GLY. Additional support for this explanation is found in animal studies which indicate that urinary excretion significantly increases its relative contribution to total GLY clearance when sGLY levels exceed 0.8 mM [significantly less than that measured in our subjects (mean range = 4.9-13.4 mM)] (Winkler et al. 1969). Urine GLY values increased rapidly in the cold divers, reaching a peak of 110.1 ± 19.2 mM at 1.5 hours postingestion, and remaining elevated (88.5 ± 19.2 mM) in the final three-hour urine sample. In contrast, Riedesel et al. (1987) reported a continuous increase in urinary GLY, with a peak of 32 mM occurring at four hours postingestion. During the four-hour postingestion period, Riedesel's subjects excreted a total of 93.5 mM GLY (or 13.8%). Likewise, McCurdy et al. (1966) found 7.5% to 13.9% of an ingested GLY dose of 1.0 to 1.27 GLY/kg in the urine of resting subjects during 2.5-hour postingestion. In close agreement, divers in our study excreted a total of 159.6 mM (or 14.3%) during an equivalent postingestion period.

Comparing the present study results with those from other GLY studies (e.g., thermoneutral, hot, or exercise) suggests that the combination of cold and immersion stress may create metabolic and physiologic conditions that diminish GLY's effectiveness as a hyperhydrating agent. Additionally, it may be that the differences in timing and administration of the GLY dosages in these two studies can explain our lack of significant effect of GLY ingestion upon water retention and diuresis.

Increased levels of sFFAs and lipid oxidation have been well documented for humans exposed to cold environments (Himms-Hagen 1972; Sink et al. 1989; Vallerand and Jacobs 1989; Wilson et al. 1969). The stimulus for this is thought

to be an increased activity of the sympathetic nervous system (catecholamine release) and stimulation of lipoprotein lipase activity in adipose tissue. In the present study, sFFA increased significantly in both groups during the three-hour cold immersion dive, approximately doubling the basal values. The mean PD sFFA concentrations (0.94 ± 0.27 mM) of the divers approximates the blood FFA concentration (0.76 ± 0.08 mM) reported following prolonged (2 hours) moderate ($44\% \text{ VO}_{2 \text{ max}}$) exercise (Ravussin et al. 1986). The slightly smaller increase in sFFA for the GLY group may result from reduced fat mobilization due to the high levels of circulating GLY (Gleeson et al. 1986; Terblanche et al. 1981).

The elevated sGLU values in the PRH sample are to be expected following the high CHO (78%) meal designed to reduce the possibility of liver glycogen depletion (and hypoglycemia) during cold water immersion. In the predive sample, sGLU had returned to normal levels from the PRH high and remained so throughout the three-hour cold immersion dive. The maintenance of normal sGLU throughout the dive can be explained by: (a) the ready availability of GLY (a prime gluconeogenic substrate); (b) the high CHO predive meal; and (c) the relatively short duration of the dive.

Urine Data

An explanation for the enhanced excretion of Na in the GLY group in the present study is not readily apparent. However, the urinary Na concentration in Riedesel's subjects at 120 minutes was also significantly higher (185%) in the GLY group than the water controls.

Daily total losses below 500 meq Na and 90 meq K are generally not considered significant. Divers in this study lost a total of only 120 meq Na and 40 meq K during the eight-hour experimental period. Shore-based Navy divers are reported to have mean 24-hour urinary losses of 194 meq Na and 66 meq K (Singh et al. 1988a). U.S. Army recruits training for 25 days in hot weather lost 140 to 254 meq Na/day and 50 to 72 meq K/day and consumed 150 to 350 meq Na/day without developing electrolyte deficits (Knochel 1977). Renal absorption and dietary intake compensate for losses in this range. Dietary intake studies of shore-based Navy divers (Singh et al. 1988b) and Navy SEALs (Goforth, unpublished data) report mean daily intakes of 194 meq Na and 74 meq K and 165

meq \pm 87 meq Na and 78 meq \pm 22 meq K, respectively. It therefore appears reasonable to conclude that the dietary intake by Navy divers of these electrolytes and renal compensation more than offsets the urinary loss and potential deficit.

In summary, it appears that consuming a single GLY dose (2 liters of water containing 1.2 g GLY/kg LBM) is ineffective in significantly decreasing body water loss (and plasma volume maintenance) under the combined stress of cold water and whole body immersion. To reduce variation in intersubject immersion diuresis, it is recommended that researchers select subjects having similar physical training and cold acclimation backgrounds for future immersion studies. Additional studies are needed to determine the effect of GLY dose concentration and timing of ingestion upon body water dynamics.

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Unclassified
SECURITY CLASSIFICATION OF THIS PAGE

| REPORT DOCUMENTATION PAGE | | | | |
|---|---|---|------------------------------|--|
| 1a REPORT SECURITY CLASSIFICATION Unclassified | | 1b RESTRICTIVE MARKINGS N/A | | |
| 2a SECURITY CLASSIFICATION AUTHORITY N/A | | 3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited. | | |
| 2b DECLASSIFICATION/DOWNGRADING SCHEDULE N/A | | 5 MONITORING ORGANIZATION REPORT NUMBER(S) | | |
| 4 PERFORMING ORGANIZATION REPORT NUMBER(S) Report No. 90-33 | | 5 MONITORING ORGANIZATION REPORT NUMBER(S) | | |
| 6a NAME OF PERFORMING ORGANIZATION Naval Health Research Center | 6b OFFICE SYMBOL (if applicable) 30 | 7a NAME OF MONITORING ORGANIZATION Chief, Bureau of Medicine and Surgery | | |
| 6c ADDRESS (City, State, and ZIP Code) P. O. Box 85122 San Diego, CA 92186-5122 | | 7b ADDRESS (City, State, and ZIP Code) Navy Department Washington, DC 20372-5120 | | |
| 8a NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research & Development Command | 8b OFFICE SYMBOL (if applicable) | 9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER | | |
| 8c ADDRESS (City, State, and ZIP Code) NNMC Bethesda, MD 20889-5044 | | 10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO 62233N PROJECT NO MM33P30 TASK NO 02 WORK UNIT ACCESSION NO 6005 DN248545 | | |
| 11 TITLE (Include Security Classification) (U) Effectiveness of Glycerol Ingestion for Enhanced Body Water Retention During Cold Water Immersion | | | | |
| 12 PERSONAL AUTHOR(S) H.W. Goforth, Jr. and D.A. Arnall | | | | |
| 13a TYPE OF REPORT Final | 13b TIME COVERED FROM 12/88 TO Aug 89 | 14 DATE OF REPORT (Year, Month, Day) | 15 PAGE COUNT 31 | |
| 16 SUPPLEMENTARY NOTATION | | | | |
| 17 COSATI CODES FIELD GROUP SUB GROUP | | 18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Hyperhydration, Glycerol, Cold, Immersion, Divers | | |
| 19 ABSTRACT (Continue on reverse if necessary and identify by block number) The efficacy of ingesting an aqueous glycerol (GLY) solution to reduce diuresis and enhance body water retention during prolonged cold water dives was tested. Six subjects were assigned to either a water treatment (WT) or glycerol treatment (GT) group. During the pre-dive period, divers drank approximately 2 liters of flavored water solutions [30 milliliters per kilogram (ml/kg) lean body mass (LBM)] with or without GLY (1.5 ml/kg LBM). Total urine output did not differ between treatments [WT = 2.1 ± 0.8 liters versus GT = 2.4 ± 0.5 liters). The amount of urinary glycerol (uGLY) collected during the hyperhydration period (prehydration to 90 minutes after hydrating) and the three-hour dive periods accounted for 4.1% and 10.3%, respectively, of the total GLY ingested. Hyperhydration with GLY (1.2 ml/kg LBM) appears ineffective in significantly reducing body water loss in divers under the stress of prolonged cold water immersion. | | | | |
| 20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS | | 21 ABSTRACT SECURITY CLASSIFICATION Unclassified | | |
| 22a NAME OF RESPONSIBLE INDIVIDUAL H.W. Goforth, Jr., Ph.D. | | 22b TELEPHONE (Include Area Code) 619-553-8379 | 22c OFFICE SYMBOL Code 30 | |